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APPLICATION OF TANSHINONE IN PRODUCING DRUGS FOR TREATING TUMORS

[Dan shen tong zai zhi bei zhi liao zhong liu yao wu zhong di ying yong]

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Claims

1. Application of tanshinone in producing drugs for treating tumors.

2. The application described in Claim 1, in that said tumors are malignant tumors.

3. The application described in Claim 1, in that said tumor treatment is inducing differentiation of tumor cells.

4. The application described in Claim 1, in that said tumor treatment is inducing tumor cell apoptosis.

5. The application described in any of Claims 1-4, in that said tanshinone is tanshinone IIA.

6. The application described in Claim 2, in that said tumor is leukemia, hepatocarcinoma, lung cancer, cerebral glioma, osteosarcoma, oophoroma, colon-rectal cancer, stomach cancer, pancreatic cancer, carcinoma of kidney, carcinoma of urinary bladder, laryngocarcinoma or nasopharyngeal carcinoma.

7. The application described in Claim 3, in that said tumor is leukemia, lung cancer, hepatocarcinoma, cerebral glioma, osteosarcoma, stomach cancer, colon-rectal cancer, carcinoma of kidney or carcinoma of urinary bladder.

8. The application described in Claim 4, in that said tumor is leukemia, lung cancer, hepatocarcinoma, cerebral glioma, osteosarcoma, colon-rectal cancer, stomach cancer, carcinoma of kidney or carcinoma of urinary bladder.

Description

This invention pertains to an application of tanshinone extract of Chinese medicine Dan Shen (red sage root) in producing pharmaceuticals, particularly to the application of tanshinone in producing drugs for treating tumors.

Dan Shen is the root of Salvia miltiorrhiza Bunge, which is a common Chinese medicine utilized for promoting blood circulation and mitigating congestion [1]. It is of a slightly cold nature and bitter taste but non-toxic. The beneficial functions include promoting blood circulation and restoring menstrual flow, removing pathogenic heat from blood and eliminating edema, and reducing anxiety and alleviating heartburn. <<Ben Cao Gang Mu>> (Compendium of Material Medica) [2] recorded "Dan Shen can treat malignant boils, psora, goiter, pyogenic infections and erysipelas, and help grow muscle". Tanshinone (Tan) is the ether or ethanol extract of Dan Shen root. Many domestic and foreign scientists have investigated the active ingredients of Dan Shen, as well as the relationship between the pharmacological effect and the chemical structures, and so far 15 ingredients have been isolated with their chemical structures elucidated (see Figure 1); these compounds are named tanshinone I (I), tanshinone IIA (II), tanshinone IIB (III), cryptotanshinone (IV), isotanshinone I (V), isotanshinone IIA (VI), isocryptotanshinone (VII), hydroxytanshinone IIA (VIII), methyl tanshinoate (IX), miltirone (X), salviol (XI), dihydrotanshinone I (XII),

Dan Shen new quinone A (XIII), Dan Shen new quinone B (XIV) and Dan Shen new quinone C (XV) [3]. Structural investigations of the 15 ingredients of Dan Shen revealed that all the active ingredients of tanshinone-type compounds in Dan Shen have ortho-quinone or para-quinone structure. Quinone compounds are easily reduced to produce diphenol derivatives, which in turn are easily oxidized to form quinone compounds, undergoing electron transfer in the process of conversion. Also, their metabolic compounds participate in many biochemical reactions in organic bodies, functioning as co-enzymes in the biological reaction by promoting or interfering in some biochemical reactions, expressing various pharmacological effects such as antibacterial activity and anti-viral activity.

Results of investigations reveal that tanshinone exhibits anti-oxidative activity; the Biophysics Institute of Chinese Medical Academy of Science investigated the influence of tanshinone IIA on the

interaction of the peroxidized products of lipids of liver cells and DNA and concluded that tanshinone IIA is a new and effective inhibitor on the interaction of the peroxidized products of lipids of liver cells and DNA, and that its protective action may have resulted through eliminating lipid free radicals and blocking the chain reaction of lipid peroxidation and inhibiting the formation of DNA adducts, thus reducing the cyto-toxicity of the latter [4].

The main clinical activity of tanshinone is cardio-vascular effect and it can inhibit arterial athrosclerosis [5], shrink myocardial infarction area and reduce myocardial oxygen consumption, showing broad inhibitory action on thrombus formation and platelet coagulation [6, 7]. Also, tanshinone IIB (III), cryptotanshinone (IV), methyl tanshinoate (IX), hydroxytanshinonoe IIA (VIII) and dihydrotanshinone I (XII) exhibit relatively potent inhibitory activities against staphylococcus aureus and drug-resistant strains thereof. Tanshinone I (I), tanshinone IIA (II), cryptotanshinone (IV) and hydroxytanshinonoe IIA (VIII) exhibit relatively potent activity against human tuberculosic bacillus ($H_{37}RV$) [3].

Tanshinone is mainly utilized in clinical treatment of coronary diseases, acne, menstrual pain, insomnia and as anti-bacterial/anti-inflammatory agent such as in treating tonsillitis, ear furuncle, pyogenic osteomyelitis and staphylococcus infection in burn, with remarkable efficacies [8].

Malignant tumors are currently posing an extreme threat to human health; the main means for treating malignant tumors are surgeries, chemotherapy and radiation therapy. The most common treatments for most solid tumors are surgeries combined with radiation therapy (radiotherapy) or with chemical therapy (chemotherapy). Chemotherapy kills tumor cells through cyto-toxic effect on tumor cells, but most chemotherapy drugs have the drawbacks of potent toxicity and serious side effects, causing many patients to terminate treatments because of intolerance to the toxicities of the chemotherapy drugs; also, the therapeutic effect may not be ideal because some tumor cells are not sensitive enough to the

chemotherapy drugs or to radiation therapy. Accordingly, developing new anti-tumor drugs having little side effects and good therapeutic effects is clinically very desirable.

Treating malignant tumors by inducing differentiation is a new pathway of tumor therapy. The main difference of treatment by inducing differentiation from chemotherapy lies in using drugs to induce the tumor cells to differentiate toward normal cells without killing the tumor cells or the normal cells, and there is little side effect such as inhibiting bone marrow function. Therefore, treatment by inducing differentiation is superior, compared with other therapeutic means, and it is receiving broad attention from medical professionals [9]. The success in treating acute promyelocytic leukemia (AFL) by inducing differentiation with all trans-retinoic acid (ATRA) in 1986 greatly promoted and advanced the progress in the basic and clinical investigations in treating malignant tumors by inducing differentiation [10]. However, the syndrome associated with retinoic acid and the rapid development of drug resistance limited the clinical application of said drug. Accordingly, searching for new highly effective and low toxic drugs for inducing differentiation for treatment of tumors is an urgent matter [11-13].

Focusing on the drawbacks of the current techniques, the objective of the present invention lies in providing tanshinone as a new means of producing drugs for treating tumors; said treatment of tumors includes, but is not limited to, killing tumor cells, inducing differentiation of tumor cells and inducing apoptosis of tumor cells.

According to one aspect of the present invention, the present invention pertains to the application of tanshinone in producing tumor treatment drugs, and said tumors include, but are not limited to, leukemia, hepatocarcinoma, lung cancer, cerebral glioma, osteosarcoma, oophoroma, colon-rectal cancer, stomach cancer, pancreatic cancer, carcinoma of kidney, carcinoma of urinary bladder, laryngocarcinoma or nasopharyngeal carcinoma.

According to another aspect of the present invention, the present invention pertains to the application of tanshinone in producing tumor treatment drugs, and said tumors include, but are not limited to, leukemia, lung cancer, hepatocarcinoma cerebral glioma, osteosarcoma, stomach cancer, colon-rectal cancer, carcinoma of kidney or carcinoma of urinary bladder.

According to still another aspect of the present invention, the present invention pertains to the application of tanshinone in producing tumor treatment drugs, and said tumors include, but are not limited to, leukemia, lung cancer, hepatocarcinoma, cerebral glioma, osteosarcoma, colon-rectal cancer, stomach cancer, carcinoma of kidney or carcinoma of urinary bladder.

Results of experimental investigations on the killing effect, differentiation-inducing effect and apoptosis-inducing effect of tanshinone are given below to describe the present invention in detail for a better understanding of the essence of the present invention.

I. Tumor-killing effect of tanshinone

In vitro investigation revealed that tanshinone exhibited killing effect on various tumor cells; 1 μg/mL tanshinone IIA exhibited killing effects on in vivo-cultured human promyelocytic leukemia cell strain HL-60, NB_4, human chronic myleogenous leukemia cell strain K562, human hepatocarcinoma cell strain SMMC-7721, human lung cancer cell strain SPC-A-1, human stomach cancer cell strain SGC7901 and human nasopharyngeal cancer cell strain CNE1.

II. Effects of tanshinone on tumor cell differentiation and apoptosis

Research results revealed that non-toxic dosages of tanshinone could induce differentiation and apoptosis of various tumor cells. The effect of 0.25-0.5 μg/mL tanshinone IIA on in vitro-cultured human promyelocytic leukemia cell strains (HL-60 and NB_4), human chronic myleogenous leukemia cell strain

(K562), human hepatocarcinoma cell strain (SMMC-7721), human lung cancer cell strain (SPC-A-1), human stomach cancer cell strain (SGC7901) and human nasopharyngeal cancer cell strain CNE1 were investigated, using 0.01% DMSO (solvent for Tan IIA) as the blank control and all trans-retinoic acid (ATRA), which is commonly utilized in inducing differentiation, as the positive control; after 5 d of drug action, the cells were collected, and the cellular morphologies were examined by optic microscope and electron microscope to calculate the rate of induction of tumor cell differentiation, rate of apoptosis and growth inhibitory rate; analysis of cell cycle was performed with flow cytometry and by assaying gene expression. Table 1 shows the results.

TABLE 1. In vitro induction effect of tanshinone on differentiation

(1) 项目 \ (2) 细胞系		HL-60	NB_4	K562	SMMC-7721	SMG7901	SPC-A-1	CNE1
(3) 药物浓度	Tan II A	1 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml
	ATRA	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml	0.5 μ g/ml
	DMSO	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
(4) 细胞形态观察		药物作用后，细胞核质比降低，核形状不规则，出现杆状核或分叶核，细胞趋于向成熟粒细胞分化 (5)	药物作用后 NB_4 细胞向终末细胞分化，其中，中晚幼粒细胞 24 %、杆状及分叶核细胞 68% (6)	中幼红以下各节段细胞占 76% (7)	药物作用后细胞排列稀疏，细胞变细、变长，大小趋向一致，细胞核质比降低，核小且核仁减少，细胞质内出现分化良好的细胞器 (8)	药物作用后细胞排列稀疏、呈长梭形或多边形，大小趋于一致、核质比增大、核仁减少，细胞趋于良性分化，部分恢复正常细胞的形态 (9)	药物作用后细胞排列稀疏，大小趋向一致，可见较多圆形细胞；电镜可见许多凋亡细胞 (10)	细胞分化和凋亡现象并存 (11)
细胞周期分析 (12)	G_0/G_1	78.7*	73.1*				75.0*	74.0*
	S	6.8*	13.3*				8.24*	14.4*
	G_2+M	14.6*	13.6*				16.8*	12.4*
(13) 诱导分化率		58.0%**	92.0%**		58.1%**	62.0%**		
(14) 凋亡率		11.4%*	21.3%*				10.1%*	33.9%*
(15) 生长抑制率		60.0%*	39.7%*	60.0%*	58.1%*	33.0%*	46.3%*	37.2%*

*与空白对照组（DMSO）比较，P<0.01 有显著差异 (16)

**与阳性对照组(ATRA)比较，P>0.05，无显著差异 (17)

Key 1 Item

2 Cell system

3	Drug concentration
4	Observation of cell morphology
5	After the drug action, the karyolitic mass ratio decreased, the nuclear morphology became irregular and slab nuclei and lobed nuclei appeared while the cells differentiated toward mature granulocytes
6	After the drug action, NB4 cells differentiated toward final cells, among them 24% mid- and late-stage myclocytes and 68% slab nuclear cells and lobed nuclear cells.
7	76% of mid-stage normoblasts and cells of latter stages
8	After the drug action, cellular arrays became loose and the cells became thin and long while the size became uniform; the karyolitic mass ratio decreased with reduced nuclear size and decreased nuclei while organelles of good differentiation appeared in cytoplasm.
9	After the drug action, cellular arrays became loose, showing long spindle shape and the size becoming uniform, while the nuclear mass increased and the nuclear number decreased; cells differentiated toward benign cells while some cells turned to normal cellular morphology.
10	After the drug action, cellular arrays became loose, and the size became more uniform while more circular cells were observed; electron microscope showed many apoptosic cells.
11	There were co-existing cell differentiation and apoptosis.
12	Analysis of cell cycles
13	Rate of induction of differentiation
14	Rate of apoptosis
15	Inhibitory rate of growth

16 Compared with the blank control group (DMSO), significant difference with $P < 0.01$

17 Compared with the positive control group (ATRA), no significant difference with $P > 0.05$

The results revealed that tanshinone could induce differentiation of tumor cells toward benign or normal cells, and the inducing effect on differentiation was not significantly different from differentiation-inducing agent ATRA currently utilized in clinical treatments. Additionally, tanshinone also exhibited an inducing effect on apoptosis of tumor cells while inducing differentiation of the tumor cells (large dosages of tanshinone exhibited cyto-toxic and cell-killing effects), suggesting that non-toxic dosages of tanshinone has tumor treatment effect.

III. Mechanism of tumor treatment effect of tanshinone

In vitro investigation revealed that tanshinone exhibited differentiation-inducing effect and anti-cancer effect, and the anti-cancer and cancer inhibitory effects are as follows:

1. Inhibition of cell proliferation and DNA synthesis

Results of flow cytometry assay revealed that tanshinone could arrest the cells at the G_0/G_1 stage and prevent them from entering the S stage, thus inhibiting the DNA synthesis and cell proliferation. Tanshinone could influence the DNA polymerase δ activity and inhibit DNA synthesis through inhibiting the expression of proliferating cell nuclear antigen (PCNA), thus inhibiting cell proliferation and inducing cell apoptosis.

2. Induction of cell differentiation and apoptosis

Research results revealed that the efficacy of an anti-cancer drug was not only determined by its interaction with specific target cells but was also determined by its ability to induce cell apoptosis, and that sensitivity of tumor cells to apoptosis was a key factor in determining chemotherapy efficacy. Tanshinone could induce apoptosis of various tumor cells, and the induction of differentiation was accompanied by cell apoptosis. Accordingly, the action mechanism of tanshinone in treating tumors may lie in its simultaneous induction of cell differentiation and induction of cell apoptosis, or in its induction of tumor cell differentiation toward matured cells and the eventual apoptosis of these matured cells.

3. Influence on DNA expression

Analysis of cellular DNA/RNA after the action of tanshinone revealed that changes occurred to the expressions of various genes of tumor cells after the action of tanshinone wherein the expression of c-myc cancer gene was reduced significantly while the expression of c-fos gene was enhanced. The expressions of c-myc and c-fos are in general considered to be closely associated with proliferation and differentiation of cells, and the amplification and over-expression of c-myc are related to the conversion of cells to malignancy and tumor formation while the decreasing expression is closely associated with the morphological change and differentiation of tumor cells; and c-fos gene is a marker for cell differentiation. Additionally, changes also occur to the expression of gene p53 and bcl-2 after the action of tanshinone. The p53 and bcl-2 genes are both closely associated with cell apoptosis; wild type p53 can promote apoptosis, while mutated p53 gene exhibits an inhibitory effect on cell apoptosis. bcl-2 gene is specifically related to cell apoptosis; bcl-2 can inhibit cell apoptosis. The expression of the bcl-2 gene in tumor cells was significantly reduced while the expression of the p53 gene was enhanced after the action of

tanshinone, suggesting that tanshinone demonstrates anti-cancer and cancer inhibitory effect through acting on the expressions of tumor differentiation and apoptosis genes.

4. Influence on tumor cell telomerase activity

HL60, K562 and APL cells were treated with 0.5 μg/mL tanshinone for 5d and 9 d; after 5 d, the inhibitory rates of Tan on the aforementioned cell telomerase activity were 30.8%, 50.8% and 37.7%, respectively (result of PCR-TRAP assay). Telomerase is thought to be a new indicator for most permanent cells and tumor cells having unlimited proliferation power. Inhibiting telomerase activity means inhibiting the growth of tumor cells, so that downgrading or inhibiting telomerase activity becomes a new idea for treating tumors. Tanshinone exhibited inhibitory effects on HL60, K562 and APL cell telomerase activities, suggesting that tanshinone possibly demonstrates its anti-tumor effect through inhibiting the telomerase activity of the tumor cells.

From the aforementioned results, the advantages of the present invention are summarized as follows:

1. The present invention discovers a new drug usage and opens up a new application field for tanshinone.

2. The tanshinone of the present invention is derived from the extract of Chinese medicine Dan Shen with little toxic side effect but with potent pharmacological effect, showing a bright prospect in pharmaceutical application.

3. The material of the present invention is abundant and inexpensive, with a simple production process, thus the prospect of industrial application is very bright.

4. The drugs produced with the substance of the present invention exhibit excellent tumor treatment efficacy, demonstrating inhibitory effects on various tumor cells through inhibiting tumor cell proliferation and inducing tumor cell differentiation and apoptosis.

5. The non-toxic dosages of the drugs produced with the substance of the present invention induce tumor cell differentiation and apoptosis; in vivo and in vitro experimental investigations revealed that non-toxic dosage of tanshinone could induce tumor cells to differentiate toward matured cells or terminal cells and inhibit the growth of tumor cells, opening up a new avenue for treating tumors.

6. Clinical investigations revealed that the tablets, capsules, injection preparations and oral preparations produced with tanshinone of the present invention exhibited remarkable treatment efficacy, showing no significant difference compared with all-trans retinoic acid currently utilized for inducing differentiation, while showing no toxic side effect, thus their clinical application has excellent prospects.

Figure 1 shows the molecular structures of the 15 ingredients of Dan Shen.

The present invention is further described with the following application examples, but they are not to be construed as limiting the present invention.

Application Example 1

In vivo anti-tumor experiments in animals

Male and female NIH mice, body weight 20-24g, were provided by the Experimental Animal Station of Si Chuan Province Anti-bacterial Industry Research Institute (Certificate No.: Chuan Shi Dong Guan No. 67). The sex of the animals was the same for each experiment, and the number of animals was > 10. Mouse hepatocarcinoma H_{22} cells were preserved for passages by Hua Xi Medical University Affiliated First Hospital Tumor Research Institute. Tanshinone IIA (Tan IIA) was a standard sample provided by Chinese Medicine Biological Product Testing Center, Lot No. 766-9204. The sample was

dissolved in 10% dimethyl sulfoxide (DMSO) and diluted to concentrations desired before application (final concentration for the experiment < 0.02%). Under an aseptic condition, ascites of hepatocarcinoma H_{22}-bearing mouse were excised and diluted to containing 1 x 10^7 tumor cells/mL, and 0.2 mL of the solution was subcutaneously inoculated in each mouse. The mice were randomly divided into 3 groups the next day: a blank control group injected subcutaneously with the same volume of DMSO solely; a positive control group injected intraperitoneally with 3 mg/kg body weight of chemotherapy drug fluorouracil (5-Fu); a test group injected subcutaneously with 20 mg/kg body weight of Tan IIA, repeated 3 times. Table 1 shows the number of animals and number of drug administrations in each group. The animals were sacrificed the day after terminating drug administration, the tumors were excised and weighed, the body weights were measured to calculate the tumor inhibitory rates, and t-test was performed in the statistical analysis. Table 2 shows the result. The tumor specimens in each group were fixed in 20% formalin, imbedded in paraffin wax and sliced, followed by HE staining and examining under an optical microscope.

TABLE 2. Inhibitory effects of tanshinone IIA on mouse hepatocarcinoma H_{22}

实验次数 (1)	药物 (2)	剂量 (3) {mg.kg^{-1}.d^{-1}}	途径次数 (4)	动物只数 (5) 始 (6)	末 (7)	体重变化 (g) (8)	平均瘤重 $\bar{x}\pm s$(g) (9)	抑瘤率 (%) (10)	P 值 (11)
1	DMSO	10 ml	SC×5	10	10	+2	1.00±0.31		
	5-Fu	30mg	Ip×4	11	11	-0.5	0.44±0.34	56.0	<0.01
	TanⅡA	20mg	SC×5	10	10	+7	0.50±0.38	50.0	<0.01
2	DMSO	10 ml	SC×5	14	14	+0.5	1.69±0.50		
	5-Fu	30mg	Ip×4	14	14	+3.5	0.91±0.27	46.2	<0.01
	TanⅡA	20mg	SC×5	12	12	+3.1	1.04±0.47	38.5	<0.01
3	DMSO	10 ml	SC×5	16	16	+4.2	1.06±0.38		
	5-Fu	30mg	Ip×4	16	16	+2	0.48±0.36	54.7	<0.01
	TanⅡA	20mg	SC×5	12	12	+4.5	0.63±0.33	40.6	<0.01

(12) 注：SC 为皮下注射；ip 为腹腔注射

(13) P<0.01 有显著差异

Key 1 Number of experiments

2 Drug

3 Dosage

4 Pathway and number of administrations

5 Number of animals

6 Beginning

7 End

8 Body weight change

9 Mean tumor weight

10 Tumor inhibitory rate

11 P value

12 Note: SC stands for subcutaneous injection; ip stands for intraperitoneal injection.

13 Significant difference, $P < 0.01$

The results revealed that tanshinone IIA exhibited significant inhibitory effect ($P < 0.01$) on mouse hepatocarcinoma H_{22}, but exerted no significant influence on the change of body weight or the general condition of the animals. Histo-pathological examinations showed various degrees of nuclear solidification and flaky necrosis of the tumor cells, hemorrhage and infiltration of inflammatory cells, more significant in the test groups compared with the control group.

Application Example 2

In vivo induction of differentiation and anti-tumor metastasis in animals

5×10^6 cells /mL of in vitro-cultured highly metastatic BALB/C mouse lung carcinoma (Lewis) cells were inoculated by injecting 0.2 mL of the solution subcutaneously in the back of BALB/C mice. The mice were randomly divided into 3 groups the next day: a blank control group injected subcutaneously with the same volume of DMSO solely; a positive control group injected intraperitoneally with chemotherapy drug fluorouracil (5-Fu, 3 mg/kg body weight); a test group administered with Tan IIA (20 mg/kg body weight by gastric perfusion) and tanshinone capsule (production method to be described later, 20 mg/kg body weight by gastric perfusion), once a day for a total of 10 d, followed by continuous breeding of the animals for 13 d until mortality appeared in the animals. The animals were sacrificed and the tumors were excised, and the body weights as well as the tumors and the lungs were measured, while the pulmonary tumor metastatic foci were observed; the tumor inhibitory rates and the inhibitory rate of tumor metastasis were calculated, and t-test was performed in the statistical analysis. Table 2 shows the result. The tumor specimens in each group were fixed in 20% formalin, imbedded in paraffin wax and sliced, followed by HE staining and examining under an optical microscope.

Preliminary results showed that tanshinone could inhibit the tumor formation of the lung carcinoma cells in mice, as well as inhibit lung metastasis of the primary tumor.

Clinical investigation in humans

Application Example 3

Drug production

Production process of tanshinone capsules

Materials: Dan Shen (Salvia miltiorrhiza Bge.) was purchased from Chengdu Chinese Medicine Company; pharmaceutical grade ethanol (Neijiang Alcohol Factory); tanshinone IIA standard sample (Chinese Medicine Biological Product Testing Center).

Methodology: Dan Shen was soaked in 12-fold 65% ethanol for 24 h, followed by diacolating at a flow rate (of the solvent) of 1/2 of the medicinal weight per h and concentrating/drying in accordance with the Pharmacopeia method, and the solid was weighed to calculate the recovery rate. Pharmaceutically permitted carriers or excipients and other optional ingredients were added to produce drugs suitable for oral administration, including capsules, flat capsules or tablets; each capsule, flat capsule or tablet contained a pre-determined quantity of tanshinone.

The content of tanshinone IIA in each capsule for clinical application was 2.5 mg tested by HPLC.

Clinical investigation

The experimental protocol and results of the pre-clinical and clinical investigations conducted at Hua Xi Medical University Affiliated First Hospital are shown below. The objective of said investigation lies in validating the efficacy of tanshinone in treating tumors and the effectiveness and (the absence of) toxic side effect in human.

Application Example 4

Treatment efficacy of tanshinone on acute promyelocytic leukemia (APL)

The treatment result of one case of promyelocytic leukemia is utilized as an example:

Methodology

1) Patient screening criteria

① Enrollment criteria:

A. Promyelocytic leukemia (M_3) patients diagnosed in accordance with FAB criteria;

B. Patients not treated with other chemotherapy drugs;

C. Patients showing no improvement or having recurrence after treatment with retinoic acid.

② Exclusion criteria: Patients with critical condition not able to complete 3-4 weeks of treatment.

2) Course of treatment

Non-blind, random, control study:

Test group: tanshinone (capsule)	30 mg tid, 2 months
Control group: retinoic acid (capsule)	20 mg tid, 2 months

3) Effectiveness

Treatment effectiveness was evaluated in accordance with the efficacy criteria stipulated at the 1987 National Leukemia Committee Meeting.

Clinical data:

CHEN Youyuan, male, 30-year-old. Visiting the Department of Hematology of Hua Xi Medical University Affiliated First Hospital as an outpatient for treatment due to headache, fatigue and dermal mucous membrane hemorrhage for half a month. Physical examination showed: general scattered hemorrhagic spots and purpuras on skin, anemic visage, no hypertrophic liver or pancreatic lymph nodes. Diagnosis by hemogram and bone marrow examination verified the case to be promyelocytic leukemia. 20 mg retinoic acid was administered orally 3 times/d for 77 d, but no improvement was observed, showing hemoglobin (Hb): 96 g/L, white blood cell (WBC): 2.3 x 10^9/L, platelet (BPC): 209 x 10^9/L, class (DC): dissociated lymph node 0.11, neutrophils 0.25, lymph nodes 0.74, bone marrow showing active proliferation of nuclear cells; promyelocytes 0.77. Drug administration was switched to 30 mg tanshinone, administered oral 3 times a d for 27 d, showing Hb: 114 g/L, BPC: 178 x 10^9/L, WBC: 4.1 x 10^9/L; after 54 d of tanshinone treatment, showing Hb: 142 x 10^9/L, BPC: 234 x 10^9/L, WBC: 9.2 x 10^9/L, DC: normal. Bone marrow smear test sowed myeloblast 0.005, promyelocyte 0.025 while physical examination showed no hypertrophic lymph nodes, liver or pancreas, achieving complete remission, and no toxic side effect was observed.

Application Example 5

In vitro investigation of induction of differentiation of APL cells by tanshinone in acute promyelocytic leukemia (APL) patients

About 5 mL bone marrow was excised from 5 cases of initial APL and single granulocyte was isolated by Ficoll, which was inoculated in a 6-well plate at 5 x 10^5/mL and treated with 5 μg/mL tanshinone IIA (Tan IIA) and 5 μg/mL all-trans retinoic acid (ATRA), respectively, followed by incubating for 7 d and observing the growth and differentiation of the cells. Table 3 shows the result.

TABLE 3. Comparison of the influences of Tan IIA and ATRA on differentiations of primary incubated APL cells

① 病例	培养天数 ②	培养条件 ③	④ 骨髓分化率（%） ⑤ 原粒细胞	早幼粒细胞	⑦ 中幼粒细胞	⑧ 晚幼粒细胞	⑨ 杆状核细胞	⑩ 嗜中性粒细胞	⑪ 诱导分化率(%)	NBT (%)
	⑥									
1	0	—	8.0	91.0	1.0					4.0
	7	C	7.0	85.0	8.0					94.0
	7	T		7.0	15.5	8.5	39.0	30.0	85.9	98.0
	7	R		4.0	27.0	2.0	42.0	25.0	88.9	
2	0	—	10.0	86.0	4.0					6.0
	7	C	6.0	82.0	12.0					90.0
	7	T		4.0	12.0	16.0	49.0	19.0	87.5	96.0
	7	R		2.0	13.0	15.0	37.0	33.0	89.6	
3	0	—	5.0	83.0	12.0					7.0
	7	C	6.0	76.0	18.0			17.5		92.0
	7	T		8.0	17.5	20.0	37.0	19.0	84.1	90.0
	7	R		4.0	15.0	14.0	48.0		88.6	
4	0	—	4.0	72.0	24.0					12.0
	7	C	2.0	66.0	32.0					90.0
	7	T	2.0	6.0	12.0	15.0	32.0	33.0	78.9	90.0
	7	R	1.0	8.0	18.0	12.5	33.0	27.5	77.6	
5	0	—		84.0	16.0					10.0
	7	C	1.0	75.0	22.0	2.0				86.0
	78	T		12.0	19.0	13.0	45.0	11.0	76.1	84.0
		R		14.0	12.0	21.0	23.0	30.0	73.8	

⑫ —：未处理 C：阴性对照 T：0.5μg/ml 丹参酮ⅡA R：0.5μg/ml 全反式维甲酸

Key 1 Case

2 Days of incubation

3 Incubating condition

4 Rate of bone marrow differentiation

5 Myeloblast

6 Promyelocyte

7 Mid-myelocyte

8 Late-myelocyte

9 Band-cell

10 Neutrophils

11 Rate of induction of differentiation

12 -: untreated; C: negative control; T: 5 μg/mL tanshinone IIA (Tan IIA); R: 5 μg/mL all-trans retinoic acid.

The result showed that Tan IIA exhibited remarkable inhibitory effect on APL cell growth in the patients with a growth inhibitory rate of 39.7% (showing no difference compared with 40.8% for ATRA); Tan IIA also significantly promoted the differentiation and maturation of APL cells with a mean induction rate of differentiation of 82.5%.

The aforementioned results of in vivo and intro investigations showed that tanshinone achieves the objective of treating tumors by inducing differentiation and/or apoptosis of tumor cells, and the efficacy is remarkable, with little side effect, thus the application prospect is bright.

The present invention is not limited to the aforementioned application examples, and those skilled in the art understand naturally that any form of modification or alteration falls in the embodiment of the present invention, so long as it is related to the new application of tanshinone in treating tumors if it is not deviated from the basic spirit of the present invention.

Description of attached figures

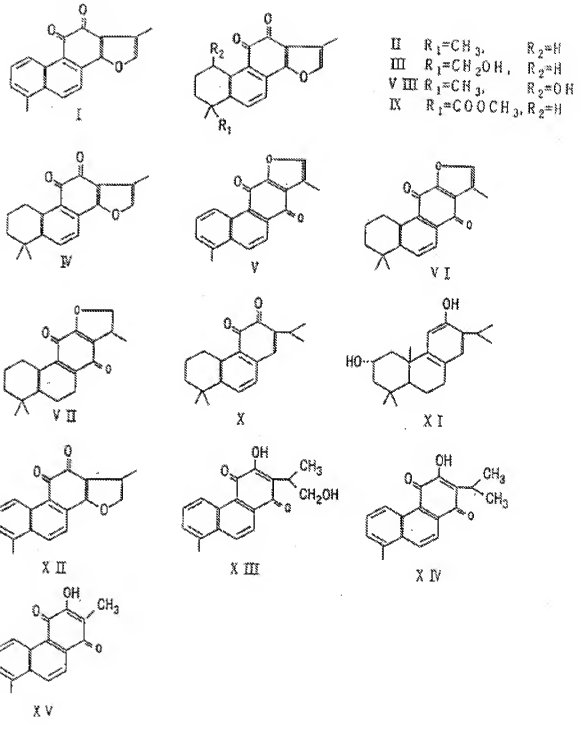

Figure 1

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